



Rancidity development during the frozen storage of farmed coho salmon (*Oncorhynchus kisutch*): Effect of antioxidant composition supplied in the diet

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ABSTRACT

A commercial diet including synthetic antioxidants (BHT–ethoxyquin mixture) (diet I) was provided to coho salmon (*Oncorhynchus kisutch*) in parallel with two diets including natural antioxidants (tocopherol isomers–rich mixture, diet II; tocopherol isomers–rosemary extract mixture, diet III). A comparative study of the rancidity development in the corresponding frozen (−18 °C) products was undertaken. When compared to fish fed with diet I, individuals corresponding to diet II showed a greater ($p < 0.05$) retention of primary (conjugated dienes and peroxides content) and secondary (anisidine and thiobarbituric acid indices) lipid oxidation compounds that led to a lower interaction compound formation (fluorescence ratio ranges: 0.33–0.50 and 0.55–0.85, for diet II and diet I individuals, respectively); likewise, a higher polyene index (1.99–2.14 and 1.72–1.97, respectively) and lower oxidised taste scores (0.0–0.6 and 0.0–2.4, respectively) were obtained. No effect ($p > 0.05$) on lipid hydrolysis development (free fatty acid formation) could be found as a result of employing different diets.

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1. Introduction

Freezing and frozen storage have largely been employed to retain fish quality before it is consumed or used in other technological processes. However, the presence in fish muscle of both a highly unsaturated lipid composition and a relevant prooxidant compound content can facilitate an important enzymatic and non-enzymatic rancidity development, this leading to sensory, physical and nutritional quality losses (Barroso, Careche, & Borderías, 1998; Erickson, 1997). To slow down such deteriorative pathways during frozen storage, previous treatments with synthetic antioxidants were successfully used, although their employment is actually not recommended because of safety concerns related to human health. In this sense, recent efforts have been focused on prior treatment with endogenous-type antioxidants (namely, tocopherol isomers) or with natural antioxidants present in plant extracts (namely, polyphenol compounds) (Kamal-Eldin & Appelqvist, 1996; Yanishlieva, Marinova, & Pokorný, 2006).

In recent years, the fishing sector has suffered from dwindling stocks of traditional species as a result of dramatic changes in their availability. This has prompted fish technologists and the fish trade to pay more attention to aquaculture as a source of fish and other seafood products. Because of its important role in the human

health, great attention has been paid to the effect of diet provided on the fish food composition. Thus, great efforts have been made to enhance the ω -3 fatty acid content in fish products by employing vegetable oil diets, including high levels of alpha-linolenic (C18:3 ω -3) acid (Chen, Nguyen, Semmens, Beamer, & Jaczynski, 2006; Visentainer, de Souza, Makoto, Hayashi, & Franco, 2005). Additionally, much research has been carried out on the effect of the diet provided on the physical and sensory properties of the processed fish, e.g. liquid holding capacity and texture (Rørå, Regost, & Lampe, 2003; Torstensen et al., 2005), colour changes (Choubert & Baccaunaud, 2006) and odour (Sérot, Regost, Prost, Robin, & Arzel, 2001).

Because of the important role of lipid oxidation development in processed fish, great attention has been paid to the endogenous antioxidant content of cultivated fish. Thus, fish farmers have included a wide range of allowed (both in the EC and the USA) synthetic antioxidants (namely, ethoxyquin, BHT and BHA) in order to enhance lipid stability in the corresponding processed food (Hertrampf & Piedad-Pascual, 2000; Southgate, 2003). However, recent efforts are focused on the replacement of synthetic antioxidants by natural ones, which may additionally provide nutritional and therapeutic effects (Frankel, 1995). Thus, diets including high contents of endogenous antioxidants have led to a partial inhibition of lipid oxidation development (Jittinandana, Kenney, Slider, Kamireddy, & Hakens, 2006; Stéphan, Guillaume, & Lamour, 1995). However, to our knowledge, comparison of the effects of diets including synthetic and natural antioxidants has not been achieved up to now.

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Among cultivated fish, coho salmon (*Oncorhynchus kisutch*), also called silver salmon, has received great attention because of its increasing production in countries such as Chile, Japan and Canada (FAO, 2007a) in parallel with important capture production in countries such as the USA, Russian Federation, Canada and Japan (FAO, 2007b). The present work focuses on the commercialisation of this species as a frozen product. In it, a commercial diet including both BHT (butyl-hydroxy-toluene) and ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) (diet I) was provided to salmon fish and compared to two other diets, one of them including a tocopherol isomer-rich mixture (diet II) and the other, a tocopherol isomers-rosemary extract mixture (diet III). A comparative study of diet effect on the fish product quality was achieved throughout frozen storage by means of rancidity development analysis.

2. Materials and methods

2.1. Experimental diets, raw material, processing and sampling

Coho salmon (*O. kisutch*) individuals used in this study were cultivated in three different tanks by EWOS Innovation Research (Colaco, Puerto Montt, Chile). Sandbed-filtered seawater (salinity range: 31.3–33.1 g kg⁻¹) was supplied to each tank over a temperature range of 11.2–12.8 °C. Feeding to satiety was carried out during the lighted period (photoperiod: 5.8–7.8 h) by employing a diet with the following general composition: protein (43.0%), fat (29.0%), moisture (7.0%), ash (6.5%), crude fibre (1.3%) and carbohydrates (13.2%). The distribution of fat composition into saturated, monounsaturated and polyunsaturated fatty acid groups was 32.5%, 27.0% and 40.2%, respectively.

Following the objectives of the work, each of the three tanks was fed with a different antioxidant composition, according to data shown in Table 1. Diet I included a relevant content of synthetic antioxidants in the meal (ethoxyquin) and in the oil (BHT); diet II provided a mixture of tocopherol isomers in both meal and oil; finally, diet III combined the presence of tocopherol isomers (in the meal) and a rosemary extract (in the oil).

Once individual salmons attained ca. 2500 g weight, 30 fish per tank were withdrawn, sacrificed by a sharp blow to the head, the gills cut and bled in a water–ice mixture, headed, gutted and kept in ice for 24 h until they arrived at our laboratory. The fish were then frozen at –40 °C in individual polyethylene bags, with hermetic sealing. After 3 days, the fish were stored at –18 °C. Frozen individuals were taken for analysis on months 0, 3, 6, 9, 12 and 18 of storage at –18 °C. From each tank under study, five different fish were independently analysed at each sampling time ($n = 5$).

2.2. Oxidised taste analysis

Oxidised taste analysis was conducted according to the quality descriptive analysis (QDA) method by a sensory panel consisting of ten experienced judges (five females and five males). Panellists were selected and trained according to International Standards

Table 1
Antioxidant composition (mg kg⁻¹ muscle) included in the different diets provided to coho salmon^a.

Antioxidant compound	Diet I	Diet II	Diet III
Total tocopherols	22.4	101	45
Ethoxyquin	19.3	2.9	2.3
BHT	3.0	ND	ND
Phenolic diterpenes	ND	ND	ND
Carnosic acid	ND	ND	18
Carnosol	ND	ND	13
Rosmarinic acid	ND	ND	ND

ND, not detected.

^a Fish supplier's data.

(ISO, 1991) in use of sensory descriptors for thawed and cooked salmon of different quality conditions.

At each sampling time, fish samples were thawed and then cooked in polyethylene bags in a water bath. The fish muscle portions were presented to panellists in individual trays and were scored individually. The panel members shared samples tested. Oxidised taste was evaluated on a non-structured linear scale with numerical scores from 0 to 10. Score 0 represents the stage of no rancidity at all, while stage 10 corresponds to the stage where no increase in rancidity is possible; score 5.0 was considered the borderline of fish acceptability. Scores among panellists were averaged.

2.3. Lipid composition analysis

The lipid fraction was extracted from the fish white muscle by the Bligh and Dyer (1959) method. Quantification results are expressed as g of lipid kg⁻¹ muscle.

Lipid extracts from the fish white muscle were converted into fatty acid methyl esters (FAME) by using acetyl chloride and analysed by GC (Perkin-Elmer 8700 chromatograph), employing a fused silica capillary column SP-2330 (0.25 mm i.d. × 30 m, Supelco Inc., Bellefonte, PA, USA) (Aubourg, Medina, & Pérez-Martín, 1996). Carrier gas used was N₂ flowing with a linear velocity of 18 cm s⁻¹. A flame ionisation detector set at 250 °C was used. Peaks were identified by comparison of their retention times with standard FAME mixtures (Larodan, Qualmix Fish; Supelco, FAME Mix). Peaks were automatically integrated, 19:0 fatty acid being used as an internal standard for quantitative analysis. The polyene index (PI) was calculated as the following fatty acid ratio: $PI = C20:5 + C22:6/C16:0$.

2.4. Lipid damage analysis

Free fatty acid (FFA) content was determined on the lipid extract by the Lowry and Tinsley (1976) method which is based on complex formation with cupric acetate–pyridine, followed by spectrophotometric (715 nm) assessment. Results are expressed as g FFA kg⁻¹ lipids.

Conjugated dienes (CD) formation was measured on the lipid extract according to the Kim and Labella (1987) method. The CD content results are expressed as absorption coefficients (AC), according to the formula: $AC = B \times V/w$, where B is the absorbance reading at 233 nm of an aliquot of the lipid extract, V denotes the aliquot volume (ml) and w is the mass (mg) of the lipid material included in the aliquot.

The peroxide value (PV) was determined on the lipid extract by the ferric thiocyanate method (Chapman & McKay, 1949). The results are expressed as meq active oxygen kg⁻¹ lipids.

The anisidine value was determined in fish muscle according to the AOCS (1993) method, based on the reaction between α - and β -unsaturated aldehydes (primarily 2-alkenals) and *p*-anisidine reagent. Anisidine value is expressed as 100 times the absorbance measured at 350 nm in a 1 cm path length cuvette from a solution containing 10 g lipid l⁻¹ reaction medium.

The thiobarbituric acid index (TBA-i) was determined according to Vyncke (1970). This method is based on the reaction between a trichloroacetic acid extract of the fish muscle and thiobarbituric acid at high temperature (95–97 °C), the resulting chromophore being measured at 532 nm. Results are expressed as mg malondialdehyde kg⁻¹ fish muscle.

2.5. Interaction compound formation

Formation of fluorescent compounds was determined with a Perkin Elmer LS 45 fluorimeter by measurements at 393/463 nm

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